Preferred orientation in an angled intercalation site of a chloro-substituted lambda- [Ru(TAP)2(dppz)]2+ complex bound to d(TCGGCGCCGA)2

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Preferred orientation in an angled intercalation site of a chloro-substituted lambda-$[Ru(TAP)_2(dppz)]^{2+}$ complex bound to $d(TCGGCGCCGA)_2$

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The crystal structure of the ruthenium DNA 'light-switch' complex Λ-$[Ru(TAP)_2(11\text{-}Cl\text{-}dppz)]^{2+}$ (TAP = tetraazaphenanthrene, dppz = dipyrido[3,2-*a'*:2',3'-*c*]phenazine)) bound to the oligonucleotide duplex $d(TCGGCGCCGA)_2$ is reported. The synthesis of the racemic ruthenium complex is described for the first time, and the racemate was used in this study. The crystal structure, at atomic resolution (1.0 Å), shows one ligand as a wedge in the minor groove, resulting in the 51° kinking of the double helix, as with the parent *lambda*-$[Ru(TAP)_2(dppz)]^{2+}$. Each complex binds to one duplex by intercalation of the dppz ligand and also by semi-intercalation of one of the orthogonal TAP ligands into a second symmetrically equivalent duplex. The 11-Cl substituent binds with the major component (66%) oriented with the 11-chloro substituent on the purine side of the terminal step of the duplex.

Introduction

In the attempt to improve the photophysical and therapeutic potential of polypyridyl ruthenium complexes, a large number of derivatives have been synthesized and their solution properties studied. The photophysical behaviour has been comprehensively reviewed [1], but much less is known about the precise DNA binding modes of this family of complexes. The 'light-switch' complexes such as $[Ru(phen)_2dppz]^{2+}$ and $[Ru(bpy)_2(dppz)]^{2+}$ have been particularly intensively studied in solution, and were finally were shown to crystallize with DNA decamer duplexes[2,3]. Although we were unable to crystallize Λ-$Ru(phen)_2(dppz)]^{2+}$ with the sequence d(TCGGCGCCGA), we were able to grow crystals with this sequence containing Λ-$[Ru(TAP)_2(dppz)]^{2+}$, showing both intercalation and semi-intercalation [4], the same sequence as is presented in this study. In these studies we have used the tetraaza (TAP) analogue instead of the phenanthroline (phen), which contains two additional ring N atoms and hence has different luminescence and hydrogen bonding properties. The differences between TAP and phen ligands in solution are due to the very different electronic effects, which make the TAP complexes photo-oxidizing [5]. They are isosteric ligands, but the TAP is much more hydrophilic, which is probably more relevant than its photooxidising properties when studying crystallogenesis. The group of DNA sequences chosen were originally chosen for study because of their interest as Holliday junction forming sequences [6], and it is still not clear whether the more complex solution equilibria associated with this group of sequences has any bearing on the success of our recent crystallisation studies.

Our interest in studying asymmetrically substituted dppz ligands came from our earlier observations that symmetry was lowered by angled intercalation, such that only one face of the dppz ligand was in contact with aqueous solution; the other was protected from water by the DNA backbone. The current theory of the 'light-switch' effect postulates that the increased luminescence lifetime of the excited state is due to the protection of the dppz phenazine N from interaction with solvent water [7]. We were therefore interested to see how asymmetric halogen substitution would modify the angled intercalation geometry, and whether there would be a site preference in an angled geometry. Therefore we here report the atomic resolution structure of Λ-$[Ru(TAP)_2(11\text{-Cl-dppz}]^{2+}$

(Figure 1a) crystallised with the d(TCGGCGCCGA) DNA duplex, which adopts the overall conformation shown in Figure 1b in the crystal lattice.

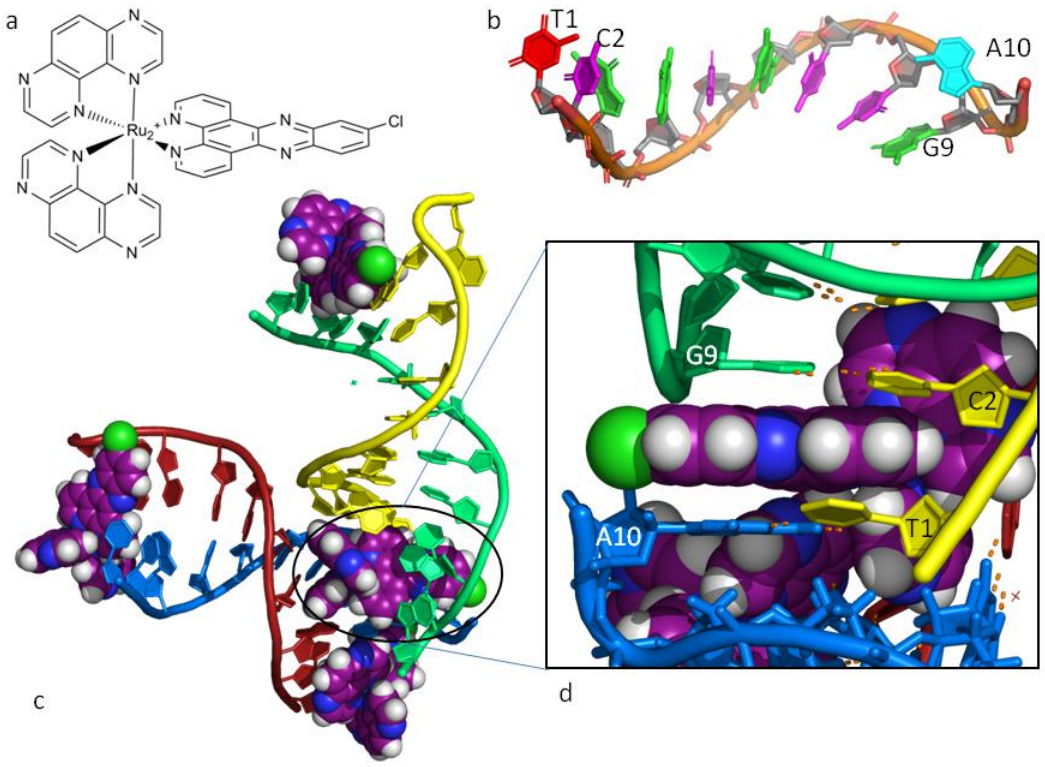

Figure 1 | Assembly of the crystal structure.

Results and discussion

The title compound is novel. It was obtained by condensation of 4-chloro-*ortho*-phenylene diamine with $[Ru(TAP)_2pd]^{2+}$, where pd is phenanthroline-5,6-dione.

Orange-red rhombohedral crystals were obtained in the presence of barium ions after a few days, and diffracted to atomic resolution, as previously observed for the parent compound. As in our previous work [2,4] the ruthenium-containing solution used for the crystallisation experiments was a racemic mixture of the chloride salt. Structural analysis revealed that only the Λ-enantiomer had bound, giving a structure with asymmetric unit consisting of a single ruthenium cation (Figure 1a), a single strand of

d(TCGGCGCCGA), in the conformation shown in Figure 1b, a barium cation, and 92 water molecules. The final resolution of the data was 1.02 Å, and the final conventional R-factor 10.3%, meaning that the structure is known with an almost 'small molecule' degree of precision. At this resolution alternative conformations will be clearly observable and can be accurately modelled. The parent structure was determined with comparable precision and resolution, being in the top 1% for precision of structures deposited in the PDB, but was remarkable in showing no sign of conformational disorder, and was accompanied by a high degree of water ordering. The introduction of asymmetry, giving a major and a minor Cl position, also generates disorder of one strand of the phosphate backbone, notably at the phosphate groups on either side of the intercalation cavity (the A_{10} residue). The second orientation of the ruthenium complex is almost exactly superimposable, except for the Cl location, and is refined at 33% statistical occupancy.

The full data collection and refinement statistics are shown in Table 1.

Data Collection	
Space group	$P\,4_32_12$
Cell Dimensions (Å)	a=b= 42.330, c = 39.630
Resolution (Å)	28.93-1.02 (1.05-1.02)*
R_{merge}	0.028(0.592)
I/σI	24.7(2.8)
Completeness (%)	98.5(99.5)
Multiplicity	6.1(6.0)
*Outer shell statistics are shown in parenthesis	

Refinement	
No. Reflections	17529
R_{work}/R_{free}	0.10/0.11
No. Atoms	
DNA	381
Ligands	147
Water	81
Average B Factors	
DNA	17.23
Ligands	14.64

Water	29.09
rmsd	
Bond lengths, (Å)	0.014
Bond Angles (°)	2.34
PDB ID	4III

The structure obtained has the gross features previously observed in the structure of Λ-$[Ru(TAP)_2dppz]^{2+}$ with the d(TCGGCGCCGA) sequence, with which the present structure is isostructural, The ruthenium complex links two duplexes, with one TAP moiety semi-intercalated at the G_3-G_4/C_6-C_7 step, and the 11-chloro-dppz intercalated at the G_9-A_{10} /T_1-C_2 step. The A_{10} base is flipped out, forming a reverse Watson-Crick basepair with a symmetry related thymine base. The flipping out created an open side to the intercalation cavity, on the purine side, and it is on this side that most of the electron density of the Cl substituent is observed. Figure 1c shows an assembly of four crystallographically equivalent nucleic acid chains, and their bound ruthenium cations, showing how the combination of intercalation by the chloro-dppz ligand and semi-intercalation by one TAP ligand creates the self-assembling three-dimensional network. This Figure shows only the major orientation of the substituted dppz chromophore, corresponding to 66% occupancy of this chlorine site. Figure 1d shows a side view of the chloro-dppz ligand, from the pyrimidine (T_1-C_2) side of the cavity. This group faces into the solvent channel of the structure, and on this side is not associated with any bound water.

There is a large solvent channel running right through the lattice in the *z* crystallographic direction, and lined by the bases of a compressed major groove. The kinking of about 50° at the G_3-G_4 step provides a convenient coordination site for a barium ion. The ion is bound to both N7 and O6 positions in the major groove, completing its 8-coordination with ordered water, which in turn lines the solvent channel.

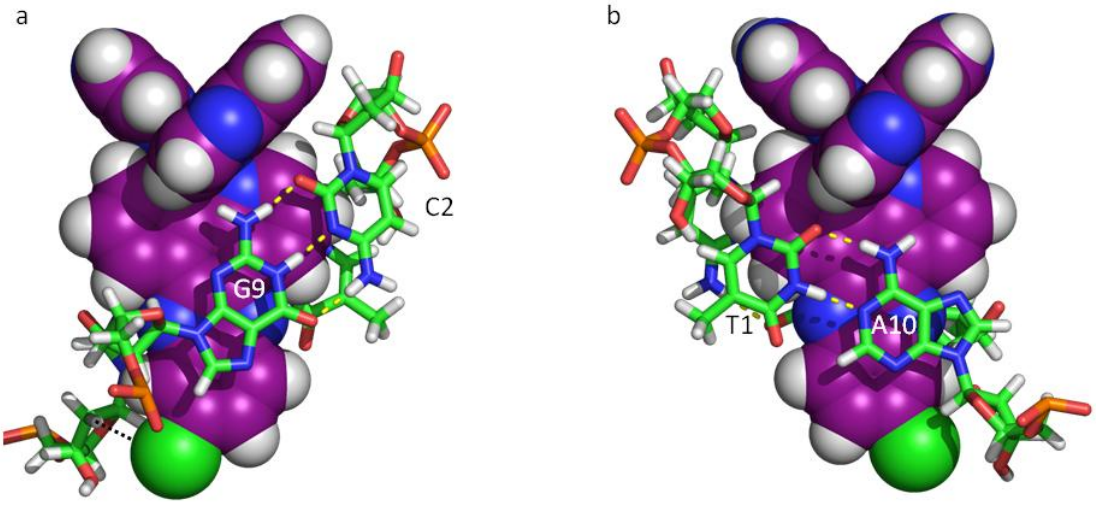

Figure 2. Stacking interactions of the chlorinated dppz chromophore

Figure 2 shows how the major orientation of the Cl substituent aligns with the basepairs of the intercalation cavity. The interpretation is complicated by the flipped out terminal adenine base, A_{10}, because it may well be that the Cl orientation is partly determined by the presence of flipping out on this side. The stacking pattern is very close to that observed in the absence of the chlorine substituent. The influence of Cl substitution is seen in two ways – in the increased level of disorder in the structure, and in the presence of a water network attached to the dppz pyrazine N atom on the Cl-substituted side of the dppz moiety. The disordered phosphate backbone at A_{10} has already been mentioned, and is associated with two distinct phosphorus positions.

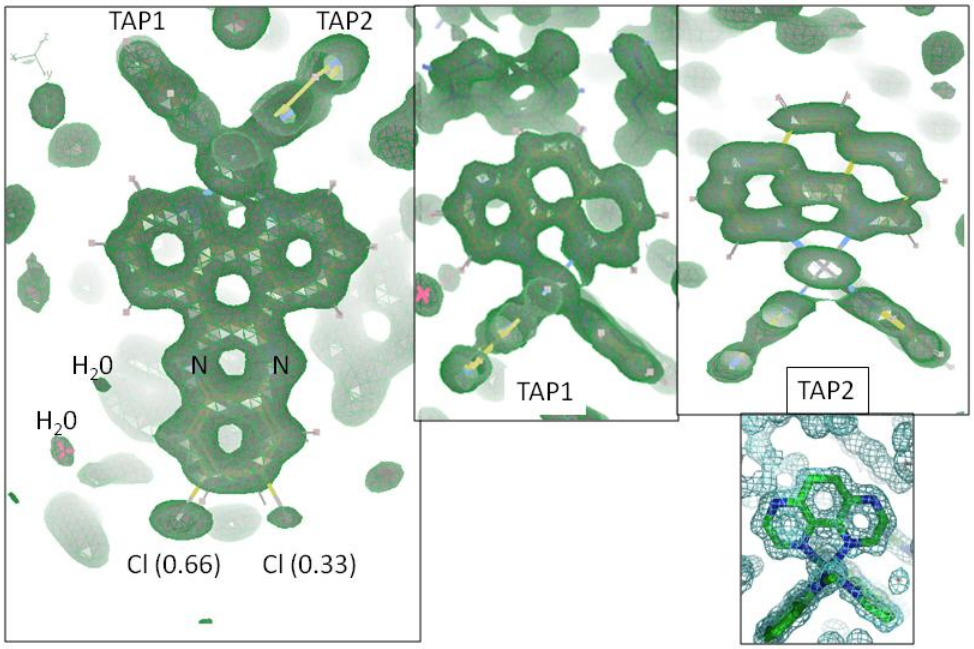

Figure 3. Quality of the electron density maps.

More subtle is the effect on the electron density of the two TAP ligands. Although chemically identical, they are distinguished by their interactions in this structure. One is semi-intercalated (TAP1), while the other is located between the minor grooves of two duplexes (TAP2). Figure 3 highlights these differences, showing the almost superimposable dppz orientations and resulting highly defined electron density. The semi-intercalated TAP1 shows similarly well-behaved density (Figure 3 centre) whereas the 'free' TAP2 ligand has electron density which clearly shows a degree of smearing-out (Figure 3 right). This feature was not observed in the previously reported parent structure [4], which is shown for comparison in Figure 3 below, right. We suggest that this might be due to the 'anchoring' effect of the semi-intercalation by TAP1, which leads to unresolved minor differences in the orientations of TAP2 which could be correlated with the two orientations of the asymmetrically substituted dppz (Figure 3).

The unsubstituted complex ions $[Ru(phen)_2dppz]^{2+}$ and $[Ru(TAP)_2dppz]^{2+}$ show luminescence (specifically ML(phen)CT and ML(TAP)CT) when intercalated into DNA, and there has been much literature discussion about the factors required for a long luminescent lifetime [1]. The best studied system, $[Ru(phen)_2dppz]^{2+}$, has phenanthroline groups as ancillary ligands on the ruthenium, in which case no luminescence was observed in aqueous solution [1]. It is widely assumed that the intense luminescence when intercalated into DNA is due to the exclusion of water from the pyrazine N of the dppz ligand. In the parent complex there are two equivalent N atoms, which if intercalated symmetrically [2] would be equally exposed to water. The lifetime would then be determined by the local DNA twist angle at the intercalation step. In the present structure, the dppz ligand is held at an angle, making the two nitrogens inequivalent. The inequivalence is further increased by the asymmetry introduced by Cl substitution. In principle there are now two possible dppz orientations and two luminescent behaviours.

Figure 4 compares the water network seen in the presence of Cl substitution and that of the unsubstituted dppz. The presence of Cl appears to promote the binding of a partly occupied water molecule to the chloro-dppz phenazine N, perhaps because the electronic effect of Cl substitution on the pyrazine N makes it a better electron pair donor.

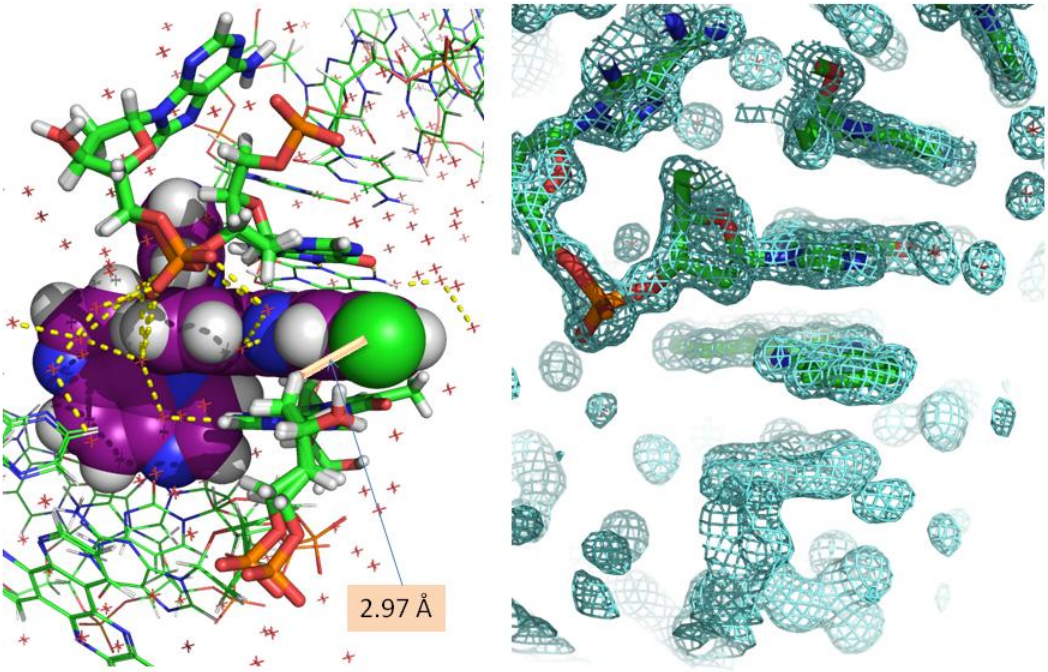

Figure 4. Water structure around the dppz ligand.

The chloro monosubstituted dppz derivative prepared as a part of this work is only the second structurally characterized asymmetric ruthenium-dppz containing complex. Recently the 11-(9-acridinyl)dipyrido[3,2-*a*:2′,3′-*c*]phenazine compound was synthesised and crystallized as the salt in the absence of DNA [8]. This compound shows a reduced DNA binding strength compared to the parent $[Ru(phen)_2(dppz)]^{2+}$. However no measurements have been made to compare an asymmetric and symmetric version of the complex, as this reduced binding could be due to the bulk of the acridinyl group. The effect of chloro substitution on the dppz group in relation to its DNA binding properties has not yet been studied. Whilst $[Ru(bpy)_2(dppz\text{-}11,12\text{-}Cl)]^{2+}$ has been synthesised and structurally characterized, no measurements were made to show its interaction with DNA [9]. The related complex $[Ru(phen)_2(dppz\text{-}11,12\text{-}Cl)]^{2+}$ has been the subject of a biophysical study, and it was found that the binding to DNA was reduced compared to $[Ru(phen)_2(dppz)]^{2+}$, with intercalation proposed as the major binding mode for the

complex[10]. This would suggest that the effect of chloro substitution is to lower the binding constant, but further studies using a range of substituents will be necessary to judge the balance between steric and electronic effects in generating the most desirable photochemical properties. We have not so far studied the effect of substitution on the TAP ligand, but nmr studies using Δ-2,9-dimethyl-1,10-phenanthroline have been used to show minor groove intercalation into $d(GTCGAC)_2$ [11], and similar minor groove binding for an achiral ruthenium complex with two octanucleotides [12].

Experimental

Preparation and characterisation of 11-chlorodipyridophenazene.-

Phenanthroline-5,6-dione was prepared by a modification of the procedure reported by Hiort, Lincoln, and Norden [13] 1,10-Phenanthroline (5 g, 27.7 mmol) was placed in concentrated sulphuric acid (30 mL), followed by sodium bromide (2.5 g), and finally concentrated nitric acid (15 mL, 70%). The mixture was refluxed at ca. 120 ^{o}C for 1 h, after which time the condenser was removed and the majority of the bromine vapour was allowed to evaporate. (ca. 30 min) The cooled mixture was then poured into cooled water (400 mL), and neutralised carefully (pH 7). The product was then heated to 80 ^{o}C, filtered hot, allowed to cool, and extracted with dichloromethane (6× 100 mL), and dried over magnesium sulphate. The filtered, dried product was found to be pure for synthesis by NMR.

The dione (260 mg, 1.24 mmol) was dissolved in ethanol (8 mL). 4-chloro-*ortho*-phenylenediamine (176.4 mg, 1.24 mmol) was dissolved in ethanol (6 mL) and added to the dione solution. A trace of *p*-toluenesulphonic acid was added. And the mixture refluxed at ca. 80 ^{o}C for 3 h. Approximately half of the solvent was evaporated at ca. 110 ^{0}C, and the cooled and the crude product filtered off. After washing with a small quantity of ethanol the product was dried under vacuum, extracted into water:ethanol 1:1 (5 mL using ultrasound and gentle heating to effect solution), filtered hot, and the residue dried under vacuum (78.7 mg). ^{1}H NMR, 700 MHz, (DMSO): δ 9.51 (m, 2H); 9.22 (m, 2H); 8.48 (d, 1H, J = 2.1 Hz); 8.42 (d, 1H, J = 9.1 Hz); 8.07 (1H, dd, J = 2.1 Hz, J = 9.1 Hz); 7.96 (m, 2H). ^{13}C NMR, 176 MHz: 153.35, 153.22, 148.73, 148.58, 142.52, 142.29,

141.83, 140.99, 136.27, 133.89, 133.81, 132.55, 131.73, 128.37, 127.44, 127.35, 125.30, 125.28, 118.76)

Preparation of 11-chlorodipyridophenazenebis(1,4,5,8-tetraazaphenanthrene)-ruthenium(II) hexafluorophosphate

Bis(1,4,5,8-tetraazaphenanthrene)ruthenium(II) dichloride (40.2 mg, 0.075 mmol) and 11-chlorodipyridophenazene (23.5 mg, 0.075 mmol) were placed in a microwave reaction tube (9 mL capacity) together with water:ethanol 1:1 (7 mL). The vessel was purged with dinitrogen for 15 minutes, and then sealed. The reaction was carried out in the microwave reactor (40 min., 140 °C, 150 W) and the vessel allowed to cool. To the cooled solution a saturated solution of ammonium hexafluorophosphate was added dropwise to precipitate the product. The solution was filtered, and the solid residue was washed with water (1×), a small quantity of ethanol (1×), and finally with a small quantity of diethyl ether. The product was dried under reduced pressure and obtained as a dark red solid (76 mg). ^{1}H NMR, 700 MHz, (DMSO): δ 9.63 (m, 2H); 9.09 (d, 2H); 9.07 (d, 2H); 8.67 (m, 4H); 8.65 (d, 1H); 8.56 (m, 1H); 8.49 (d, 2H); 8.38 (m, 4H); 8.22 (d, 1H); 7.94 (m, 2H). ^{13}C NMR, 176 MHz: 156.05, 155.92, 150.87,150.70, 150.37, 150.18, 150.16, 149.71, 145.22, 145.09, 142.71, 142.47, 142.46, 141.23, 140.03, 137.86, 135.02, 134.98, 134.03, 133.09, 132.91, 131.97, 130.46, 130.37, 128.64, 128.44. (26 of the expected 28 signals are resolved. 2 peaks overlap even at 700 MHz). It was converted to the chloride salt using Amberlite resin for the crystallisations.

Crystallization, data collection, structure solution and refinement

The crystallization was performed, using the sitting drop method, by adding 1µl of 3mM rac-$[Ru(TAP)_2(11\text{-chloro-dppz})]^{2+}$ to a drop containing 1 µl of 1 mM $d(TCGGCGCCGA)_2$, and 6ul of 12mM spermine, 10% 2-methyl-2,4-pentanediol, 40mM sodium cacodylate pH 6.3, 80mM NaCl and 20mM $BaCl_2$. This was equilibrated against 1ml of 35% 2-methyl-2,4-pentanediol. Orange crystals, approximately 150x150x150 µm in size and only containing Λ-$[Ru(TAP)_2(11\text{-Cl-dppz})]^{2+}$, appeared after 7 days at room temperature.

The 90° of data were collected in 900 frames with a 0.1° oscillation at Diamond Light Source on beamline I02 using radiation of wavelength 0.8266 Å at 100 K. The data were processed using xia2 [14] using XDS[15] and SCALA [16], in the CCP4 suite [17], to

give 18496 unique reflections to 1.02 Å resolution. The structure was solved by molecular replacement using Phaser[18] and model 3QRN from the Protein Data Bank, and was updated using Coot[19]. The structure was refined using Refmac version 5.6 [20] to give a final R_{work} of 0.10 and R_{free} of 0.11. 5% of reflections were used for the R_{free} test. The model and experimental data are deposited in the Protein Data Bank with ID 4III.

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